recites "at least 10^5 transformants/μg DNA." The language of the amendment is supported at page 5, lines 17-19. The amendment adds no new matter.

Objection to the Specification:

The specification is objected to because it contains an embedded hyperlink at page 11. Applicants have herein amended the paragraph containing the hyperlink to remove browser-executable code.

Rejections under 35 U.S.C. §112, second paragraph:

Claim 22 is rejected for lack of proper antecedent basis for the term "said glass-forming matrix material." Claim 22 is amended herein to change the dependency from claim 18 to claim 21, which recites a glass-forming matrix material. Applicants submit that the amendment is sufficient to overcome this ground of rejection.

Claim 23 is rejected for lack of proper antecedent basis for the term "said at least one carbohydrate." Applicants submit that the amendment of claim 22 to recite "at least one" carbohydrate, and the amendment of claim 23 to depend from claim 22, rather than from claim 19 is sufficient to overcome this ground of rejection.

Claims 28 and 63 are rejected because they recite the trademark "ficoll™." The Office Action states that because a trademark identifies the source of goods, and not the goods themselves, the trademark does not identify or describe the goods associated with the trademark. Applicants respectfully disagree.

First, Applicants submit that ficoll has a Chemical Abstracts Service (CAS) registry number (26873-85-8), which means that it is a chemical compound of definite structure. Ficoll is a polymer of α-D-Glucopyranoside, β-D-Fructofuranosyl with (chloromethyl)oxirane (see attached Exhibit A). Second, Applicants submit that a recent search of the U.S.P.T.O. patent database revealed 80 issued patents in which the term "ficoll" appears in the claims (see attached Exhibit B). In a number of these, e.g., U.S. 6,437,101, U.S. 6,465,425 and US 6,432,629, among others, the term "ficoll" appears in a Markush listing of polysaccharides, dextrans and glycols having similar properties.

(Applicants also note that the Bronshtein et al. application cited as prior art also recites ficoll in its claims.) Applicants submit that because ficoll has a CAS registry number and because the term is frequently used in the claims of issued patents, the term is definite within the requirements of 35 U.S.C. §112, second paragraph.

In view of the above, Applicants respectfully request the withdrawal of the §112, second paragraph rejection of claims 22, 23, 28 and 63.

Rejections under 35 U.S.C. §102:

The claims are novel over Bronshtein et al.

Claims 1-3, 5-8, 16-41, 47-55 and 58-74 are rejected under 35 U.S.C. §102(b) as being anticipated by Bronshtein et al., WO 99/27071. The Office Action states that Bronshtein et al. "teach a method of generating storage stable XL10 gold competent cells, E. coli gram negative cells that are generated by exposure to buffer containing $CaCl_2$, that comprises drying cells above freezing for at least 8 hours in a vacuum under non-atmospheric pressure." Applicants respectfully disagree.

Applicants submit that the Broshtein et al reference does not teach a method that comprises drying competent cells at a temperature above freezing. Specifically, the Bronshtein et al. reference does not teach the drying of competent cells. While one strain of E. coli dried in the Bronshtein et al. reference is XL10 Gold™, the cells are not taught to be competent before or after they are dried. On page 10, line 23 to page 11, line 2, the Bronshtein et al. reference describes the steps taken to dry the cells. In that protocol, a frozen suspension of the XL10-Gold™ cells are thawed, cultured in NZYM broth overnight, and an aliquot of this culture is used to inoculate another culture that is then grown to a specified OD, centrifuged, and re-suspended in preservation solution consisting of 25% sucrose and 25% fructose in MRS broth. These cells are then dried under vacuum. Applicants submit that the XL10-Gold™ cells were not taught to be competent when thawed, and that cells cultured and treated in this manner are not competent. The Declaration of Alan L. Greener under Rule 132 submitted herewith supports the conclusion that the XL-10 Gold™ cells dried in the Bronshtein et al.

reference were not competent before or after drying. Specifically, Dr. Greener, who developed the E. coli strain marketed by Stratagene as XL-10 Gold, states that

> "While some strains of cells may be genetically superior to others with regard to competence, even these strains require some treatment, such as with $CaCl_2$, to render them competent. Further, culture of cells treated to induce competence results in the loss of competence, because the cultured cells repair the damage to their membranes upon proliferation. Thus, when cells, whether originally competent or not , are expanded in culture and dried without some treatment to establish (or re-establish) competence, the cells resulting from the expansion culture are not competent. That is, *competence is not an inherent property of cells, including XL-10 Gold™ cells*." (Attached Rule 132 Declaration of Alan L. Greener; emphasis added)

Dr. Greener then states that XL-10 Gold™ cells grown and treated as described in the Bronshtein et al. reference prior to drying would not be competent without a step to render them competent. Because the cells dried in the Bronshtein et al. reference were not competent, the Bronshtein et al. reference cannot anticipate the claimed invention, which in all instances requires the cells to be competent. Applicants respectfully request the withdrawal of the §102(b) rejection of claims 1-3, 5-8, 16-41, 47-55 and 58-74 over Bronshtein et al. Also, because it does not teach drying competent cells, Bronshtein et al. does not anticipate new claims 81 and 82, which both require "generating storage-stable *competent* cells."

The claims are novel over Jessee et al.

Claims 1-3, 6-12, 15-16, 19-21, 23-28, 31, 43, and 45-46 are rejected under 35 U.S.C. §102(b) as being anticipated by Jessee et al., WO 98/35018. The Office Action states that the Jessee et al. reference teaches "a method of generating storage stable competent cells that comprises drying cells above freezing for at least 8 hours in vacuum under a non-atmospheric pressure," and that "the competent cells are preferably E. coli and are made competent in a buffer containing $CaCl_2$." The Office Action further states that the reference teaches that the dried cells are storage stable for at least 45 days at temperatures ranging from 4°C to –80°C with a transformation efficiency of at least 10^5.

The Office Action thus concludes that the claims are anticipated by this reference. Applicants respectfully disagree.

Applicants submit that the Jessee et al. reference teaches freeze-drying, a process that differs significantly from the claimed invention. According to the definition set forth in Hawley's Condensed Chemical Dictionary (14th Edition, revised by Richard J. Lewis, Sr., John Wiley & Sons, Inc., New York, New York, 2001), "freeze drying" is:

> "A method of dehydration or of separating water from biological materials. The material is first frozen and then placed in a high vacuum so that the water (ice) vaporizes in the vacuum (sublimes) without melting, and the nonwater components are left behind in an undamaged state." (Hawley's Condensed Chemical Dictionary, 14th Edition, page 515; Exhibit C)

Applicants submit that the method taught by Jessee et al. meets this definition. Specifically, at page 9, lines 27 to 29, Jessee et al. states that "Prior to lyophilization, the cells are frozen at about ⁻20°C to ⁻180°C, preferably at about ⁻80°C to about ⁻180°C, most preferably about ⁻80°C. Also, at page 10, Jessee et al. states:

> "The cells are then lyophilized by techniques which are well known in the art. Lyophilization is a process by which ice and/or moisture is removed from frozen cells by sublimation under vacuum at low, subzero temperatures (e.g., -40° to –50°C). Any residual moisture associated with the "dried" preparation is then removed by gradually raising the temperature, resulting in evaporation." (Jessee et al., page 10, lines 9-13)

In view of the above, Applicants submit that the Jessee et al. method, involving freezing the cells solid at subzero temperatures and application of high vacuum, meets the definition of "freeze-drying."

Applicants submit that the claimed invention does not encompass freeze-drying. More specifically, where freeze drying requires that the sample be frozen prior to drying, so that ice sublimates off, the present invention does not require freezing of the sample, and the specification actually teaches away from the sample being frozen immediately before drying. Claim 1 as amended does not involve freeze drying as taught by Jessee et al., because the claim requires that the cells "are suspended in the liquid state

immediately prior to said drying." The language of the amendment is supported in the instant specification at page 13 which states:

> Competent cells which have been previously frozen can be used, as well as competent cells which are freshly made (e.g., less than 2 hours old) and have been stored at –20°C to 4°C. However, preferably, *the cells are not frozen immediately prior to drying* (i.e., at least 1 minute prior to drying)." (specification page 13, lines 15-18; emphasis added)

Thus, the cells are "suspended in the liquid state" immediately prior to drying. Also on page 13, the instant specification states:

> "Drying can be performed using standard drying apparatuses known in the art as lyophilizes (sic), sublimates, Speedvacs, and the like. Freeze-drying apparatuses can be modified for use in the process *(e.g., by not drying in the presence of dry ice, or by setting a temperature control to a temperature above freezing, i.e., such as room temperature or above).* ***The cells themselves are never freeze-dried.***" (page 13, lines 25-29; emphasis added)

Because, according to the specification, the cells are never freeze dried, the cells cannot remain frozen at the time the vacuum is applied, because application of vacuum to frozen cells results in freeze drying. The limitation that "the cells are suspended in the liquid state immediately prior to said drying" means then, that the cells are not frozen immediately prior to drying. Applicants note that the claimed invention permits a sample to have been frozen at some point before drying (see claim 1 and the passage at page 13, lines 15-18), as long as the sample is not in the frozen state immediately prior to drying, which would lead to freeze drying.

Applicants note that new claims 81 and 82 added herein are also novel over Jessee et al. because they require that the "cells are not frozen immediately prior to said drying" (claim 81) and that the "cells are not frozen at least one minute prior to said drying" (claim 82). Because Jessee et al. only teaches freeze-drying, the reference cannot anticipate these new claims.

The claims are novel over Barnea et al.

Claims 1-3, 6-17, 21, 24, 29, 43, 45 and 46 are rejected under 35 U.S.C. §102(e) as being anticipated by Barnes et al. (U.S. Patent Application No. 2002/0081565). The Office Action states that the Barnea et al. reference teaches a method for generating storage stable competent cells that are also transformed with exogenous DNA. The Office Action also states that Barnea et al. teaches that cells were lyophilized at 0°C for 10 hours, 5°C, 10°C, 15°C, 20°C and 25°C for 30 minutes each in CB-I buffer with sucrose or trehalose, and that the cells exhibit a transformation efficiency of at least 10^5 transformants/μg DNA. The Office Action thus concludes that the Barnea et al. reference anticipates the claimed invention.

First, Applicants submit that Barnea et al. is not prior art over the claimed invention. Applicants submit herewith the Rule 131 Declarations of inventors Alan Greener and James Jolly establishing conception and reduction to practice of the claimed invention prior to October 30, 2000. Specifically, the attached Declarations show that competent cells had been dried at a temperature greater than freezing before that date. The dried cells retained viability and competence. For example, the Declarations include notebook entries from before October 30, 2000 showing: A) electrocompetent E. coli cells dried at 4°C, ramping up to 30°C – viability and electrocompetence were maintained; B) electrocompetent E. coli cells dried at 30°C for 2 hours – viability was similar to control cells, and electrocompetence was approximately 2-fold higher than for control cells; and C) chemically competent E. coli cells (XL-10 Gold) dried at 30°C – viability and competence were maintained. In no case were the cells freeze-dried. The cells were always suspended in the liquid state immediately prior to the drying.

Applicants submit that the attached Rule 131 Declarations demonstrate (before October 30, 2000) not only the drying of competent cells at a temperature greater than freezing, but that the cells are suspended in the liquid state immediately prior to drying, i.e., the cells not frozen immediately prior to the drying. This satisfies the limitations of claim 1 as amended and therefore demonstrates the prior invention of that invention claimed in claim 1.

In view of the Declarations of Drs. Greener and Jolly, Applicants submit that Barnea et al. is not prior art over the presently claimed invention.

Applicants further submit that not only is Barnea et al. not prior art, but the reference does not teach all limitations of the claimed invention. Specifically, Barnea et al. does not teach "a method for generating storage-stable competent cells, which comprises drying competent cells at a temperature greater than freezing so as to generate storage stable competent cells, wherein the cells are suspended in the liquid state immediately prior to the drying." Barnea et al. only teaches freeze drying, i.e., first freezing the cells, and then applying vacuum to sublimate off the ice. That is, Barnea et al. does not teach that the competent cells are suspended inn the liquid state immediately prior to drying. Rather, Barnea et al. specifically teaches freeze-drying. See, for example, page 2, paragraph [0018] which states "It has been discovered that cells rendered competent or partially competent may be produced in a process wherein the competent cells are freeze-dried. Paragraph [0019] also states "The invention, in its preferred form, is a process for producing freeze-dried competent cells that may be stored for extended periods of time or shipped as a dry powder at temperatures between about $0^{o}C$ to about $8^{o}C$, more preferably at temperatures between about $0^{o}C$ to about $4^{o}C$, most preferably shipping on wet ice."

As discussed above in regard to the Jessee et al. reference, the invention of claim 1 as amended is not anticipated by a reference that teaches only freeze-drying. Therefore, Applicants submit that the Barnea et al. reference, even if it were prior art, which it is not, would not anticipate the claimed invention because the reference does not describe all elements of the claimed invention. Applicants respectfully request the withdrawal of the §102(e) rejection of claims 1-3, 6-17, 21, 24, 29, 43, 45 and 46 over Barnea et al.

Applicants note that new claims 81 and 82 added herein are also novel over Barnea et al. because they require that the "cells are not frozen immediately prior to said drying" (claim 81) and that the "cells are not frozen at least one minute prior to said drying" (claim 82). Because Barnea et al. only teaches freeze-drying, the reference cannot anticipate these new claims. Further, as supported by the Rule 131 Declarations

of Alan Greener and James Jolly, the Barnea et al. reference is not prior art over the invention of new claims 81 and 82.

Rejections under 35 U.S.C. §103:

Claims 47-63 and 78-80 are not obvious over Jessee et al. in view of Bronshtein et al.

Claims 47-63 and 78-80 are rejected under 35 U.S.C. §103(a) as obvious over Jessee et al. in view of Bronshtein et al. The Office Action states that Jessee et al. teaches the use of glass-forming cryoprotectants that include carbohydrates such as trehalose, sucrose, sorbitol, PVP, and ficoll wherein cells exhibit at least 10^5 transformants/μg DNA, but also states that the Jessee et al. reference does not teach how to determine, nor does it give Tg values for the cryprotectants used. Regarding Bronshtein et al., the Office Action states that the reference teaches the use of Tg values in storage determination. Bronshtein et al. is said to teach a secondary drying under vacuum in the range of 0-100°C for periods of time sufficient to increase the glass transition temperature to a point above the selected storage temperature within the range of 0°C to 70°C, and that this is necessary to provide long term shelf preservation of biological suspensions in the glass state. The Office Action states "therefore, the Tg desired can be achieved by manipulating drying time and temperature; a Tg of a matrix-cell mixture greater than 15°C is achieved by drying the matrix-cell mixture at 25°C for 40+ hours (figure 2). This can be reasonably accomplished for any choice of protectant used for cell drying." The Office Action concludes that it would have been obvious to one of ordinary skill in the art to utilize Tg values for determining the time and duration of drying due to the success in the art of these temperatures for predicting stability of biological material under long term storage. Applicants respectfully disagree.

Claims 47-63 relate to: "a composition comprising a glass-forming matrix material and competent cells wherein the glass transition temperature (Tg) of the matrix-cell mixture is greater than 15°C" (claims 47-55); "a composition comprising a glass-forming matrix material and competent cells wherein the glass transition temperature (Tg) of the matrix-cell mixture is greater than 15°C wherein the transformation efficiency of the cells is at least 10^5 transformants/μg DNA" or "at least or 10^6 transformants/μg

DNA" (claims 56 and 57, respectively); and "a composition comprising a glass-forming matrix material and competent cells wherein the glass transition temperature (Tg) of the matrix-cell mixture is greater than 15°C, wherein the glass-forming matrix comprises at least one carbohydrate" (claims 58-63).

Applicants submit that Bronshtein et al. teaches boiling samples at reduced atmospheric pressure to generate a foam (see, for example, Bronshtein et al. claim 1, and page 7, lines 17-22). In contrast, Jessee et al. teaches freeze-drying (freezing the samples solid, then applying vacuum) with gradual increases in temperature. Applicants submit that it would not be possible to apply the Bronshtein et al. boiling method to the drying method of Jessee, which is optimized to competent cells. Solid samples do not boil to generate a foam under vacuum, as taught to be necessary by Bronshtein et al., but rather sublimate directly from the solid. The aspects of the two disclosures suggested to be combined by the Office Action cannot be so combined without changing the principle of operation of one or both references. A combination of references requiring a change in the basic principles of operation of a reference cannot form the basis of an obviousness rejection. In re Ratti, 270 F.2d 810, 813 (C.C.P.A. 1959). As such, the combination of cited references does not render obvious the claimed invention.

Finally, Applicants submit that while Bronshtein et al. teaches the determination of Tg values, the values shown in the cited Figure 2 are for the Tg of a solution of sucrose and raffinose (1:1) without cells. Applicants submit that solutions of solutes other than sucrose and raffinose will have different patterns in the relationship between Tg and drying time and temperature. Thus, it is not correct when the Office Action asserts that a desired Tg "can be reasonably accomplished for any choice of protectant used for cell drying" on the basis of Bronshtein et al.'s teachings exemplified in Figure 2.

In view of the above, Applicants submit that the combination of Bronshtein et al. with Jessee et al. does not render obvious the invention of claims 47-63.

With regard to methods claims 78-80, which are drawn to methods of producing a recombinant polypeptide, Applicants submit that because the combination of Jessee et al. and Bronshtein et al. suggested by the Office Action is not operable to generate a

composition that maintains the competence and viability of competent cells, the combination of references would not be useful to generate a method to produce a recombinant polypeptide in which competence and viability of the cells are necessary traits. Applicants submit that the invention of claims 78-80 is therefore not obvious over the combination of Jessee et al. and Bronshtein et al.

In view of the above, Applicants submit that the combination of Bronshtein et al. with Jessee et al. does not render obvious the invention of claims 47-63 and 78-80. Applicants respectfully request the withdrawal of the rejections under §103(a) over Jessee et al. in view of Bronshtein et al.

The Office Action states on page 8 that claims 4, 42, and 75-77 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. Applicants wish to thank the Examiner for pointing out allowable material, but respectfully submit that in view of the amendments and remarks set forth herein, the subject claims are allowable as written, so no amendments to claims 4, 42 and 75-77 are made herein. Further, applicants note that on page 1 the Office Action lists claims 4, 42, 44 and 75-77 as allowed, while the paragraph on page 8 of the Office Action omits claim 44 from this list. Clarification is kindly requested.

In view of the above, Applicants submit that all issues raised in the Office Action are addressed herein. Applicants respectfully request the reconsideration of the claims.

Respectfully submitted,

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Kathleen M. Williams, Ph.D.
Registration No. 34,380
Customer No.: 27495
PALMER & DODGE LLP
111 Huntington Avenue
Boston, MA 02199-7613
Telephone: (617) 239-0451
Telecopier: (617) 227-4420

Version of Amendments Marked to Show Changes:

In the Specification:

- On page 11, replace the paragraph at lines 24-29 with the following replacement paragraph:

Additional methods of generating competent cells are described in: Kushner, In: *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering*, Elsevier, Amsterdam, pp. 17-23 (1978); Norgard, et al., Gene 3: 279-292 (1978); Jessee, et al., U.S. Patent No. 4,981,797; and on the world wide web at protocol-online.net/molbio/DNA/transformation.html [at http://www.protocol-online.net/molbio/DNA/transformation.html], the entireties of which are incorporated by reference herein.

In the Claims:

1. (Amended) A method for generating storage-stable competent cells, which comprises drying competent cells at a temperature greater than freezing so as to generate storage-stable competent cells, wherein said cells are suspended in the liquid state immediately prior to said drying.

22. (Amended) The method of claim [18] 20, wherein said glass-forming matrix material comprises [a] at least one carbohydrate.

23. (Amended) The method of claim [19] 22, wherein said at least one carbohydrate comprises a saccharide.

57. (Amended) The composition of claim 47, wherein the transformation efficiency of said cells comprises at least [10^5] 10^6 transformants/μg DNA.

81. (New) A method for generating storage-stable competent cells, which comprises drying competent cells at a temperature greater than freezing so as to generate storage-stable competent cells, wherein said cells are not frozen immediately prior to said drying.

82. (New) The method of claim 81 wherein said cells are not frozen at least one minute prior to said drying.